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Evaluation of a *Treponema pallidum* Western Immunoblot Assay as a Confirmatory Test for Syphilis

ROBERT E. BYRNE,* SHARON LASKA, MOLLY BELL, DORIS LARSON, JACK PHILLIPS, AND JOHN TODD

Pandex Division, Baxter Diagnostics, 909 Orchard Street, Mundelein, Illinois 60060

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Tests for the detection of antibodies to *Treponema pallidum* are recommended for the confirmation of reactive nontreponemal test results and the accurate diagnosis of syphilis. The present-day use of Western blot (immunoblot) technology for the diagnosis of retroviruses prompted the development and evaluation of a Western blot assay with whole-cell *T. pallidum* as the antigen. The assay detected antibodies in syphilitic serum or plasma from dilutions of specimens incubated overnight with test strips. A test was considered positive when at least three of four major antigens having molecular masses of 15.5, 17, 44.5, and 47 kDa were detected. The Western blot assay had 93.8% sensitivity and 100% specificity for clinically defined samples. The Western blot assay was compared with double-staining fluorescent treponemal antibody absorption [FTA-ABS (DS)], which had a sensitivity and a specificity of 91.7 and 92.0%, respectively. Dilution series studies of syphilis-positive specimens indicated that the Western blot assay has an endpoint of reactivity at least 3 to 4 serial dilutions greater than that for FTA-ABS (DS). Overall, the >95% agreement between the Western blot assay and FTA-ABS (DS) for clinically defined specimens indicates that the sensitivity of the Western blot assay is equal to or greater than that of FTA-ABS (DS). The Western blot assay demonstrated no false-positive or equivocal reactivities for nonsyphilitic specimens, including normal specimens (both plasma and serum), biological false-positives, and specimens with elevated gamma globulin or antinuclear antibody. We conclude that the high sensitivity and specificity of the *T. pallidum* Western blot assay, together with its simplicity and objectivity, make it a good confirmatory test for syphilis.

Physicians need to use both clinical and serological information to accurately diagnose syphilis. Nontreponemal tests, such as the Venereal Disease Research Laboratory slide and the rapid plasma reagin (RPR) 18-mm circle card, have long been used to test blood samples from patients for routine physicals, prenatal examinations, venereal disease clinics, hospital admissions, and blood bank screening. Since the incidence of false-positive reactions with nontreponemal tests may be as high as 70% in some populations, confirmation by a treponemal test is strongly recommended to establish or rule out a diagnosis of syphilis (14).

The microhemagglutination assay for *Treponema pallidum* (MHA-TP) and the fluorescent treponemal antibody absorption test (FTA-ABS) are the two most common confirmatory treponemal tests, with FTA-ABS being the procedure most commonly used in the United States (14). In addition, various investigators have developed Western blot (immunoblot) procedures using a lysate from *T. pallidum* (5, 11). This test has been suggested as a possible alternative to either FTA-ABS or MHA-TP (5).

A reactive FTA-ABS or MHA-TP result is widely accepted as evidence of past or present syphilis. However, FTA-ABS, when improperly performed, presents various problems of interpretation for the laboratorian or physician. FTA-ABS is a subjective test with additional difficulties caused by improper training of technologists, poor quality of reagents, and improper microscope illumination (6, 14). In addition, minimally reactive (1+) samples represent a significant diagnostic problem for the physician. In those cases, FTA-ABS must be viewed as equivocal (10).

There has been considerable discussion concerning the sensitivity and specificity of FTA-ABS (14). The sensitivity of FTA-ABS, as is true for the other confirmatory tests, is lowest in primary syphilis specimens, as many as 20% of which may be nonreactive (14). FTA-ABS is more sensitive in later stages, with 99 to 100% reactivity during the secondary stage and 95 to 100% during the latent and late stages (14). A number of investigations have shown that false-positive FTA-ABS reactions may occur in (i) patients with increased levels of gamma globulin or antinuclear antibody (ANA) (8, 12, 16, 22), (ii) pregnant patients (2), and (iii) patients with autoimmune hemolytic anemia (12).

Although currently used confirmatory tests are very good, improvements in health care can be achieved by either improving existing tests or introducing new methods for the confirmation of reactive nontreponemal test results. Existing confirmatory tests, as well as the Centers for Disease Control (CDC), use serum as the only specimen type for syphilis testing. New methods that can test both plasma and serum will provide hospital and public health laboratories with increased efficiency, better quality of testing, and lower blood collection costs.

The purpose of the present study was to compare a *T. pallidum* Western blot assay with FTA-ABS. Special emphasis was placed on testing clinically defined specimens, biological false-positives, problem samples, normal donor samples, dilution series, high-risk-patient samples, and plasma versus serum as a specimen type.

MATERIALS AND METHODS

***T. pallidum* antigen.** *T. pallidum* Nichols was maintained in adult male New Zealand White rabbits by testicular passage (3). Infected rabbits were individually housed, maintained at 16 to 18°C, and given antibiotic-free food and water. Each treponemal suspension was prepared from infected rabbit testicles 10 to 14 days after inoculation (3).

* Corresponding author.

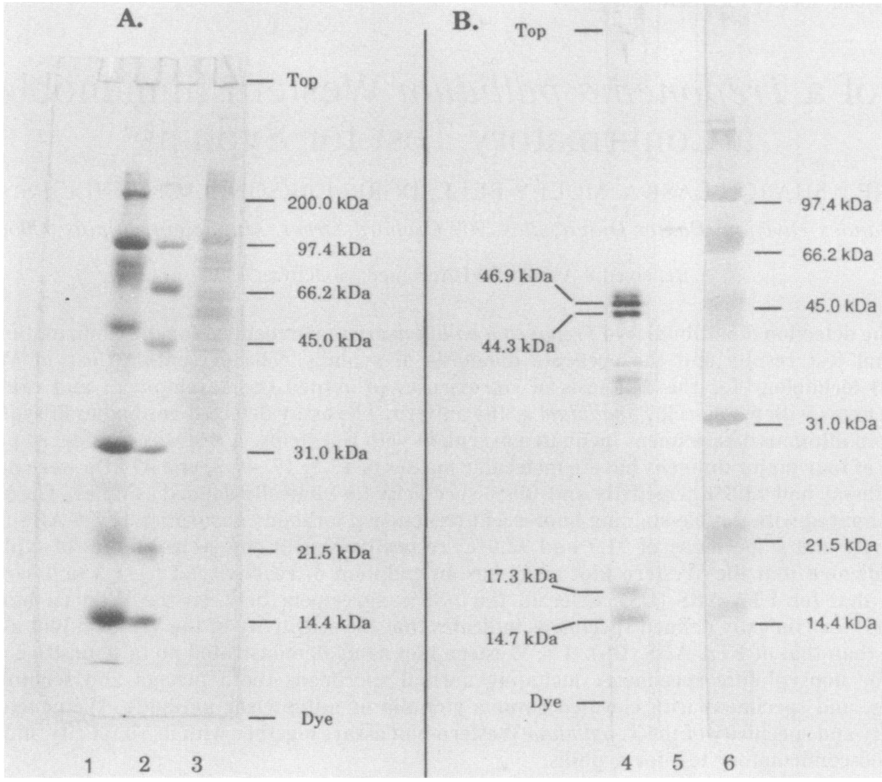

FIG. 1. SDS-PAGE patterns and Western blot patterns of the major immunoreactive proteins in *T. pallidum*. A portion of the SDS–12% polyacrylamide gel containing panel A (lanes 1 to 3) was excised and stained for protein with Coomassie brilliant blue R-250, and that containing part B (lanes 4 to 6) was electrotransferred to a polyvinylidene difluoride membrane and immunostained. Lanes: 1, rainbow molecular mass markers (14 to 200 kDa); 2, low-molecular-mass markers (14 to 100 kDa); 3, *T. pallidum* preparation; 4, *T. pallidum* blot with a syphilis-positive plasma sample; 5, *T. pallidum* blot with a normal plasma sample; 6, Coomassie brilliant blue R-250-stained blot of low-molecular-mass markers.

For use as a working stock of antigen, treponemal suspensions were diluted to 2×10^7 organisms per ml, as determined by dark-field microscopy counting (4).

Human blood specimens. Serum and plasma specimens were obtained from the following sources: CDC panels from Sandra Larsen, Center for Infectious Diseases, CDC (Atlanta, Ga.); normal blood donor samples from the Milwaukee Blood Bank (Milwaukee, Wis.); RPR-reactive serum samples from Vital Blood Products (Los Angeles, Calif.); clinically defined primary syphilis serum specimens from Howard Brown Memorial Clinic (Chicago, Ill.); and FTA-ABS-reactive specimens for dilution series from Baxter Screening Lab (Roundlake, Ill.).

Specimen testing methods. Double-staining FTA-ABS [FTA-ABS (DS)] was performed with a commercial kit in accordance with manufacturer's directions (Zeus Scientific, Inc., Raritan, N.J.). Reactive samples were visualized with rhodamine-labelled anti-human immunoglobulin G. Fluorescein-labelled antitreponemal antigen was used to counterstain. FTA-ABS (DS) was done with a multiwell *T. pallidum* antigen substrate slide. Included controls for nonspecific staining by conjugates were the reference diluents of Zeus-phosphate-buffered saline (zPBS) alone and sorbent (standardized extract of *T. phagedenis* biotype Reiter) alone, which gave no distinct fluorescence, and the following control specimens, with the expected relative fluorescence readings in parentheses: reactive control diluted 1:5 with zPBS (4+), reactive control diluted 1:5 with sorbent (3+ to 4+), minimally reactive control diluted with zPBS (1+), nonspecific control diluted 1:5 with zPBS (2+), and nonspecific control diluted 1:5 with sorbent (−). Test specimens were diluted 1:5 in sorbent before incubation with antigen on the slide. The reactivities of unknown specimens were scored on the basis of the *T. pallidum* rhodamine fluorescence of these controls. Specimens with test readings of >1+ were reactive, and those with test readings of either − or − to <1+ were nonreactive. Minimally reactive (1+) specimens were retested. Specimens were always tested in a blind fashion. CDC panels were tested by FTA-ABS (DS) at the CDC by a standard method (18). ANA testing was performed with a commercial indirect antibody kit in accordance with package insert instructions (Bartels Division, Baxter Diagnostics, Inc., Issaquah, Wash.). Rheumatoid factor (RF) and RPR 18-mm circle card tests were performed with commercial card agglutination assays in accordance with the manufacturer's directions (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting. Separation of *T. pallidum* polypeptides was performed with a Laemmli discontinuous buffer system (9), a 12% separating gel (pH 8.8), and a 4.5% stacking gel (pH 6.8). In particular, 0.200 ml of whole-cell *T. pallidum* antigen in 2% sodium dodecyl sulfate (SDS)–0.125 M Tris (pH 6.8)–10% glycerol–1% 2-mercaptoethanol–0.02% pyronin Y tracking dye was boiled for 5 min in a water bath. The sample was applied as two 0.100-ml aliquots to two prepar-

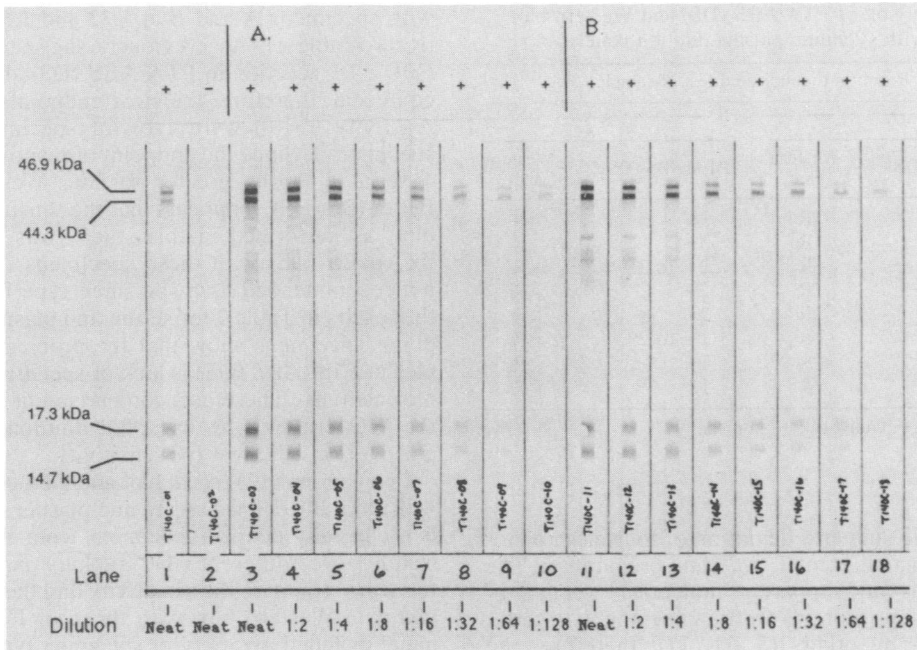

FIG. 2. Immunoreactivity of serially diluted syphilitic specimens in the *T. pallidum* Western blot assay. Lanes: 1, syphilis-positive control; 2, syphilis-negative control; 3 to 10, specimen A; 11 to 18, specimen B. Specimens were diluted in negative plasma, and the dilution for each test strip is indicated below each lane. Above each test strip is the interpreted Western blot reactivity (+, reactive; −, nonreactive).

ative wells (64 mm each) and electrophoresed at a constant current until the tracking dye reached the bottom. The apparent molecular weights of *T. pallidum* proteins were estimated by extrapolation from a standard plot of the logarithm of the molecular weights versus the electrophoretic mobilities of Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.)-stained standard proteins (Bio-Rad Laboratories, Richmond, Calif.) and rainbow molecular weight markers (Amersham Corp., Arlington Heights, Ill.) by the procedures described by Weber and Osborn (23). The separated proteins were electrotransferred in a tank apparatus (Hoeffer, San Francisco, Calif.) to a polyvinylidene difluoride membrane (Millipore, New Bedford, Mass.) in a Tris-glycine system as described by Towbin et al. (21). After transfer, the membrane was blocked in a solution of powdered nonfat dry milk dissolved in blotting buffer (BB) (0.02 M sodium phosphate, 0.15 M NaCl [pH 7.4], 0.1% NaN_3) as described by Johnson et al. (7). The membrane was rinsed briefly in BB, cut into strips (3 by 120 mm), and either used immediately or stored at −20°C. Western blot strips were placed in troughs of an incubation tray (Bio-Rad) and rewet with nonfat dry milk in BB. The appropriate volume of test specimen was added to each trough to achieve a 1:100 dilution of original serum or plasma and incubated overnight at room temperature with gentle agitation. After incubation, the strips were washed four times with BB-Tween 20 (Sigma) (15) and incubated for 1 h with alkaline phosphatase-conjugated goat anti-human immunoglobulin G plus immunoglobulin M (Jackson Immunoresearch, Westgrove, Pa.) in BB-nonfat dry milk solution. The strips were washed three times with BB-Tween 20. Development of the strips was accomplished with the nitroblue tetrazolium–5-bromo-4-chloro-3-indolyl phosphate system in substrate buffer as described by Blake et al. (1), and the strips were allowed to air dry on blotting paper in the dark. A test was considered positive when at least three of the following four bands were present: 15.5, 17, 44.5, and 47 kDa (see Results). A test was considered negative when no band or only one of the above-listed specific bands was present, while a test was considered equivocal when two of the above-listed specific bands was present. Positive and negative controls were run with each group of specimens.

Methods of statistical analysis. The comparison between the Western blot assay and FTA-ABS (DS) was analyzed by contingency-table (2 × 3) analysis and a chi-square goodness-of-fit test for differences in proportion among positive, negative, and equivocal reactivities. When a P value was >0.05, the observed differences among positive, negative, and equivocal interpretations were not significant (19). Ninety-five percent confidence intervals were calculated by the secant method (20).

RESULTS

Identification of the major immunoreactive *T. pallidum* protein antigens by Western blot analysis. The apparent molecular weights and antigenicities of the major polypeptides of *T. pallidum* were characterized by comparing the Coomassie brilliant blue R-250-stained SDS-PAGE patterns with the corresponding immunoblot patterns. The apparent molecular weights of the identified proteins were estimated by extrapolation from a plot of the logarithm of the molecular weights versus the electrophoretic mobilities of standard proteins (Fig. 1A, lanes 1 and 2, and Fig. 1B, lane 6). The major Coomassie brilliant blue R-250-stained proteins ranged in size from approximately 33 to >200 kDa (Fig. 1A, lane 3). In contrast, the Western blot pattern of *T. pallidum*, when reacted with antibodies of syphilitic [strong FTA-ABS (DS) reaction] sera or plasma, exhibited major bands of 14.7 kDa (±0.6 kDa), 17.3 kDa (±0.7 kDa), 44.3 kDa (±0.6 kDa), and 46.9 kDa (±0.8 kDa) (Fig. 1B, lane 4). The apparent molecular weights of the major immunoreactive proteins

TABLE 1. Comparison of FTA-ABS (DS) and Western blot reactivities with syphilitic plasma dilution panels

Dilution	Result in the indicated test of sample: A: FTA-ABS (DS)[a]	A: Western blot[b]	B: FTA-ABS (DS)[a]	B: Western blot[b]
Neat	4+	R	3+	R
1:2	4+	R	3+	R
1:4	3+	R	2+ to 3+	R
1:8	2+ to 3+	R	2+	R
1:16	2+	R	2+	R
1:32	1+	R	1+ to 2+	R
1:64	−	R	1+	R
1:128	−	R	1+	R

[a] 2+ to 4+, reactive; 1+, equivocal; −, nonreactive.
[b] R, reactive.

were the mean ± the standard deviation of four electrophoretic runs, each with a different *T. pallidum* preparation. The SDS-PAGE patterns and apparent molecular weights of these immunodominant proteins were in keeping with those previously reported by others (5, 11, 17); therefore, the major immunoreactive proteins will henceforth be labelled 15.5, 17, 44.5, and 47 kDa. Additional immunoreactive proteins of 30, 33 to 35, and 38 to 40 kDa were also detected. Normal serum (i.e., nonsyphilitic) was nonreactive with the 47-, 44.5-, 17-, and 15.5-kDa bands, although a minor reaction with a protein of approximately 80 to 90 kDa was observed in the example shown in Fig. 1B, lane 5). Additional reactions with a variety of minor bands were also seen on occasion with other nonsyphilitic sera (data not shown). It is interesting to note that major immunoreactive proteins which corresponded to the 17- and 15.5-kDa antigens and which were not readily visible by Coomassie brilliant blue R-250 staining in Fig. 1A, lane 3, were detected by silver staining (data not shown).

Sensitivity of the *T. pallidum* Western blot assay. *T. pallidum* Western blot strips were incubated overnight with two syphilitic specimens which were each serially diluted in negative plasma and examined for immunoreactivity with the four major antigens (Fig. 2). A positive Western blot was defined as reactivity with at least three of the four major immunoreactive bands. Both specimens A and B exhibited reactivity with all four of the major antigens, even at a 1:128 dilution. In contrast, FTA-ABS (DS) yielded 1+ reactions with specimens A and B at 1:32 and 1:64 dilutions, respectively (Table 1). As previously suggested by Larsen et al. (10), a 1+ reaction in FTA-ABS (DS) should be considered equivocal; therefore, the clear endpoints of FTA-ABS (DS) reactivity (>1+) were 1:16 for specimen A and 1:32 for specimen B. Since the endpoint of reactivity was at least 3 to 4 serial dilutions greater for the Western blot than for FTA-ABS (DS), it appears that the sensitivity of the Western blot, as performed in this study, was better than that of FTA-ABS (DS) with these specimens. Although plasma is not recommended as a specimen type for FTA-ABS (DS), the results in Table 2 for serum and plasma testing of normal donor specimens show that the problem with plasma samples in FTA-ABS (DS) is lack of specificity, rather than loss of sensitivity. Therefore, comparison of FTA-ABS (DS) and the Western blot by use of a dilution series in negative plasma is a valid test for sensitivity.

Comparison of Western blot and FTA-ABS (DS) reactivities with clinically defined serum and plasma specimens. Seventy-five clinically defined specimens from the CDC and representing three different CDC syphilis panels were evaluated to further compare the sensitivity and the specificity of the *T. pallidum* Western blot with those of FTA-ABS (DS). The panel included a variety of specimen types, such as normal human donors; disease states other than syphilis; primary, secondary, and latent syphilis; biological false-positives; and Pinta (a nonvenereal spirochetal infection caused by *T. carateum*). Results obtained with CDC panel 2 are presented in Fig. 3 (30 specimens) and were typical of the results for the other two panels tested. Table 3 (45 specimens) shows results obtained with CDC panels 1 and 3. The Western blot assay and FTA-ABS (DS) were in complete agreement for every specimen shown in Fig. 3. All specimens classified as normal (lanes d to f), disease states other than syphilis (lanes g to j), and biological false-positives (lanes 7 to 9) were correctly identified as negative by both tests. In particular, both tests correctly identified primary syphilis specimens (lanes l to p), secondary syphilis specimens (lanes q to y), and latent specimens (lanes 2 to 4 and lane 6) as positive. Both tests also failed to identify as positive a primary syphilis specimen (lane k; also negative with RPR and Venereal Disease Research Laboratory tests and MHA-TP) and a latent syphilis specimen (lane 5; positive with RPR and Venereal Disease Research Laboratory tests). It is interesting to note that none of the four major *T. pallidum* antigen bands was observed by the Western blot assay with these false-negatives.

TABLE 2. Comparison of Western blot and FTA-ABS (DS) reactivities with primary syphilis specimens, normal donor specimens, ANA- or RF-reactive specimens, and specimens from i.v. drug abusers

Specimen category	No. tested	No. of specimens showing the following FTA-ABS (DS) and Western blot results, respectively[a]: + and +	+ and −	− and +	− and −	E and +	E and −	% Agreement[b]	P value[c]
Primary syphilis	40	36	0	0	3	1	0	97.5	$0.25 < P < 0.50$
Normal donor	13[d]	0	2	0	9	0	2	69.2	$0.025 < P < 0.050$
	15[e]	1	1	0	8	0	5	60.0	$0.025 < P < 0.050$
ANA or RF reactive	10	0	2	0	5	0	3	50.0	$0.025 < P < 0.050$
i.v. drug abusers	19	9	0	1	6	2	1	78.9	$0.10 < P < 0.25$

[a] FTA-ABS (DS) reactivity: +, >2+; −, <1+; equivocal (E), 1+. Western blot reactivity: +, reactive; −, nonreactive.
[b] Percent agreement = [(number of samples that were + and + and that were − and −)/total number of samples] × 100.
[c] *P* value for chi-square goodness-of-fit test for differences in proportion.
[d] Serum.
[e] Plasma.

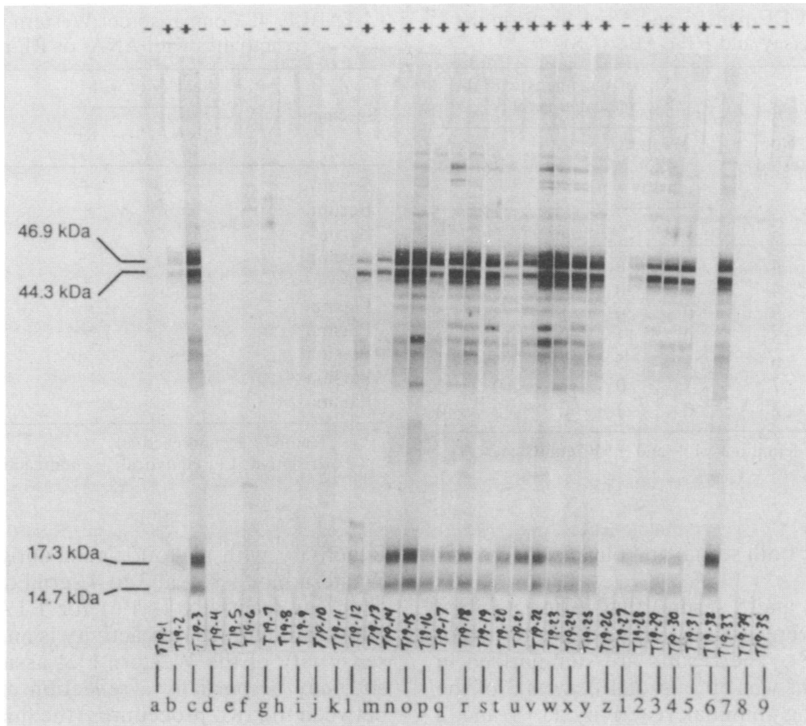

FIG. 3. *T. pallidum* Western blot reactivity with CDC syphilis performance panel 2. A reactive (+) or nonreactive (−) Western blot interpretation is indicated above each test strip. The clinical diagnosis of each CDC specimen and the FTA-ABS (DS) reactivity provided by the CDC are indicated in parentheses below. Reactivity in FTA-ABS (DS) was scored 1+ to 4+, with increasing intensity. Nonreactivity in FTA-ABS (DS) was indicated as N. Strips: a, syphilis-negative control (N); b, low-reactivity syphilis-positive control (2+); c, high-reactivity syphilis-positive control (3+); d, normal (N); e, normal (N); f, normal (N); g, disease other than syphilis (N); h, disease other than syphilis (N); i, disease other than syphilis (N); j, disease other than syphilis (N); k, primary syphilis (N); l, primary syphilis (3+); m, primary syphilis (4+); n, primary syphilis (4+); o, primary syphilis (4+); p, primary syphilis (3+); q, secondary syphilis (4+); r, secondary syphilis (4+); s, secondary syphilis (4+); t, secondary syphilis (2+); u, secondary syphilis (4+); v, secondary syphilis (4+); w, secondary syphilis (3+); x, secondary syphilis (4+); y, secondary syphilis (3+); z, syphilis-negative control (N); 1, low-reactivity syphilis-positive control (2+); 2, latent syphilis (3+); 3, latent syphilis (4+); 4, latent syphilis (3+); 5, latent syphilis (N); 6, latent syphilis (4+); 7, biological false-positive (N); 8, biological false-positive (N); 9, biological false-positive (N).

The results shown in Table 3 indicate that the Western blot assay and FTA-ABS (DS) were in close agreement, except for the following discordant results: the Western blot assay detected all nine primary syphilis specimens, whereas FTA-ABS (DS) was nonreactive with one; one latent syphilis specimen was Western blot nonreactive and classified as minimally reactive (1+) by FTA-ABS (DS); and two of seven biological false-positive specimens were FTA-ABS (DS) minimally reactive or equivocal, whereas all seven were classified correctly as nonreactive by the Western blot assay. Both tests were reactive with the two Pinta specimens (Table 3), a result which was expected because of the similarity of the antigens of the two pathogenic treponemes (13).

Clinical diagnosis is the reference method used to compare the performance of the Western blot assay with that of FTA-ABS (DS). Overall, when the data for the 73 CDC specimens (excluding the two Pinta specimens) shown in Fig. 3 and Table 3 are analyzed together, the Western blot assay performed with a sensitivity of 93.8% (45 of 48 positive specimens) and a specificity of 100% (25 negative specimens, including 7 normal specimens, 8 with disease states other than syphilis, and 10 biological false-positives). The corresponding sensitivity and specificity for FTA-ABS (DS) were 91.7 and 92.0%, respectively (44 of 48 positive specimens and 23 of 25 negative specimens).

Performance of the *T. pallidum* Western blot assay and FTA-ABS (DS) with primary syphilis specimens, normal donor specimens, ANA- or RF-reactive specimens, and specimens from i.v. drug abusers. In addition to the CDC panels previously described, 90 specimens from other sources were tested to compare the *T. pallidum* Western blot assay with FTA-ABS (DS). Table 2 displays the Western blot and FTA-ABS (DS) reactivities for primary syphilis specimens, normal donor specimens, ANA- or RF-reactive specimens, and specimens from intravenous (i.v.) drug abusers.

Forty clinically defined primary syphilis serum specimens (20 treated and 20 untreated) were assayed by the Western blot assay and FTA-ABS (DS). The percent agreement was 97.5% (39 of 40) at a confidence level of 95%. The one discrepant sample displayed all four bands in the Western blot assay and had 1+ reactivity in FTA-ABS (DS). P values of >0.25 and <0.5 indicated that there was no significant difference between the observed results of the Western blot assay and FTA-ABS (DS).

The agreement between the Western blot assay and FTA-ABS (DS) for 13 random normal donor serum specimens was 69.2% (9 of 13). Percent agreement for 15 plasma specimens from normal donors was 60%. A high percentage of the discrepant samples (7 of 10) had 1+ reactivity in FTA-ABS (DS). P values of >0.025 and <0.05 indicated that the observed difference between the Western blot assay and

TABLE 3. Reactivity of CDC panel 1 and 3 specimens in the Western blot assay and FTA-ABS (DS)

Category	No. tested	No. of specimens with the indicated result[a]: Western blot assay –	Western blot assay +	FTA-ABS (DS) –	FTA-ABS (DS) +	FTA-ABS (DS) E
Normal blood donors	4	4	0	4	0	0
Other[b]	4	4	0	4	0	0
Primary syphilis	9	0	9	1	8	0
Secondary syphilis	13	0	13	0	13	0
Latent syphilis	6	1	5	0	5	1
False-positives	7	7	0	5	0	2
Pinta	2	0	2	0	2	0

[a] See Table 2, footnote a, for definitions of – and +; E, equivocal (1+).
[b] Diseases other than syphilis.

TABLE 4. Comparison of Western blot and FTA-ABS (DS) reactivities with ANA- or RF-reactive specimens

Specimen	Reactivity with[a]: ANA	Reactivity with[a]: RF	Result in: FTA-ABS (DS)[b]	Result in: Western blot assay[a]
Serum	–	+	2+	–
Serum	–	+	–	–
Serum	+	–	1+	–
Serum	+	–	–	–
Serum	–	+	–	–
Plasma	–	+	2+	–
Plasma	+	–	1+	–
Plasma	+	–	1+	–
Plasma	+	–	–	–
Plasma	+	–	–	–

[a] +, reactive; –, nonreactive.
[b] 2+, reactive; 1+, equivocal; –, nonreactive.

FTA-ABS (DS) results for both serum and plasma specimens was significant.

Ten samples (5 plasma and 5 serum) that tested positive for either ANA or RF were assayed by the Western blot assay and FTA-ABS (DS). The results are summarized in Tables 2 and 4. Agreement was 50% (5 of 10), with 3 of the discrepant samples having minimal reactivity (1+) and 2 having positive reactivity (2+) in FTA-ABS (DS). There was a significant difference between the observed results of the Western blot assay and FTA-ABS (DS), as evidenced by P values of >0.025 and <0.05.

Nineteen serum samples from i.v. drug abusers were tested by the Western blot assay and FTA-ABS (DS). Percent agreement was 78.9% (15 of 19), with three of the four discrepant samples having minimal reactivity (1+) in FTA-ABS (DS). P values of >0.1 and <0.25 were calculated for this group of samples. Therefore, there was no significant difference between the observed results of the Western blot assay and FTA-ABS (DS).

DISCUSSION

The need for a sensitive and specific confirmatory test for syphilis is well established (14). *T. pallidum* Western blot technology has been used as a confirmatory tool for research purposes by various investigators (5, 11) and has been suggested as an alternative to FTA-ABS (DS) (5). We have developed a Western blot assay that detects antibodies to *T. pallidum* in serum or plasma. The purpose of this study was to compare the Western blot assay with FTA-ABS (DS), the confirmatory test that is commonly used in the United States.

The four major protein antigens which reacted in the *T. pallidum* Western blot assay with antibodies in syphilis-positive plasma had molecular masses similar to those previously reported (5, 17). The 46.9-, 44.3-, 17.3-, and 14.7-kDa bands have been reported by a consensus of various other investigators as 47-, 44.5-, 17-, and 15.5-kDa bands, respectively (17). The molecular masses cited by other investigators fall within 2 standard deviations of the means calculated for our Western blot assay. These four bands have also been shown nonreactive with syphilis-negative serum and plasma.

The sensitivity of the Western blot assay was studied with clinically diagnosed syphilis specimens. First, two syphilis-positive specimens were serially diluted in negative plasma and tested by the Western blot assay and FTA-ABS (DS). Reactivity with all four major antigens was present in the Western blot assay at 3 to 4 serial dilutions greater than the endpoint reactivity (>1+) for FTA-ABS (DS). This two-dilution difference in reactivity is an indication of the greater sensitivity of the Western blot assay with these specimens and may be partially a reflection of the dilution difference between the two procedures [the final dilution for FTA-ABS (DS) is 1:5, and that for the Western blot assay is 1:100]. Second, more than 80 clinically diagnosed syphilis specimens were analyzed by the Western blot assay and FTA-ABS (DS). The Western blot assay correctly interpreted 93.3% of clinically defined syphilis specimens as positive. In contrast, FTA-ABS (DS) was less sensitive (90.1%), partly because of a lack of reactivity with primary syphilis specimens. The Western blot- and FTA-ABS (DS)-nonreactive specimens from CDC panel 2 (Fig. 3) were a primary specimen and a latent specimen that were also nonreactive in MHA-TP. They showed none of the four specific bands in the Western blot assay. The lack of reactivity in all three treponemal tests can probably be explained by either a low antibody titer or clinical misdiagnosis. Because the percent agreement between the Western blot assay and FTA-ABS (DS) with clinically defined reactive specimens was very high (>95%) and chi-square analysis showed no significant difference in performance with syphilitic sera, we conclude that the sensitivity of the Western blot assay is equal to or greater than that of FTA-ABS (DS).

The specificity of the Western blot assay was analyzed with biological false-positive samples, normal donor samples, and problem samples containing increased levels of gamma globulin or ANA. The Western blot assay was nonreactive with all biological false-positive samples, all ANA- or RF-reactive samples, and all but one normal donor sample. In contrast, FTA-ABS (DS) demonstrated a lower specificity (65.8%). Chi-square analysis of the normal donor samples and the ANA- or RF-reactive samples showed that there was a significant difference between the observed results of the Western blot assay and FTA-ABS (DS). The nonspecific nature of the FTA-ABS (DS) test noted in this study has been reported by other investigators (2, 6, 8, 12, 14, 16, 22). In summary, the specificity of the Western blot assay appears to be better than that of FTA-ABS (DS) for the number of samples tested.

A drawback of FTA-ABS (DS) is the equivocal reactivity (1+) observed with some samples. We found 14 samples (7 serum and 7 plasma) that fell into this category, corresponding well with the results for CDC panels 1 to 3 as well as reports from other investigators (6, 10, 14). This study showed that the frequency of equivocal results with the Western blot assay was lower than that with FTA-ABS (DS). First, the Western blot assay did not show any indications of equivocal reactivity (two of the four antigen bands were present) with normal donor samples, biological false-positive samples, or ANA- or RF-reactive samples. Samples representing all stages of syphilis were reactive with at least three of the four major bands, with various intensities. Second, serial dilutions of syphilis-positive samples showed a proportional decrease in reactivity with all bands, not a selective decrease in reactivity, indicating that even weakly reactive samples reacted with all bands. Therefore, reactivity with only one or two of the four major antigens in a syphilitic sample is rare and should be interpreted as negative or equivocal, respectively.

A limitation of commercial confirmatory tests for treponemal antibodies is the stipulation that a test specimen be serum. Part of the present study was done to demonstrate that the Western blot assay can be performed with both plasma and serum. No false-positive reactions were seen in the Western blot assay with normal donor plasma specimens or ANA- or RF-reactive plasma specimens. With a limited sample size, therefore, it appears that plasma is an acceptable specimen type for the Western blot assay.

The present study showed no equivocal reactions in the Western blot assay (two of four antigens were present) that could cause problems of interpretation. Specimens with such reactions most likely exist, and future studies will reveal their presence. An interesting experiment will be a study of the reactivity of these specimens in the Western blot assay at higher concentrations of serum or plasma than were used in the present study. It may be possible to identify infection in the central nervous system by Western blot analysis of cerebrospinal fluid. In future studies, we will continue to evaluate sensitivity with specimens representing different stages of syphilis and to test the specificity of the Western blot assay with other possible nonspecific reactive specimens, such as those from Lyme disease. Even though the nonspecific staining or background of Western blot strips is low with negative specimens, the use of recombinant *T. pallidum* antigens should improve background even further.

It is well documented that *T. pallidum* shares common epitopes with other treponemes, such as *T. phagedenis* biotype Reiter (see, e.g., Lukehart et al. [11] and Norris et al. [17]). These cross-reactions apparently occur mainly with the flagellar and other nonspecific proteins in the 30- to 40-kDa range. Pathogen-specific epitopes are reported to be within this molecular mass range, but we have chosen to base our criterion of reactivity on the 15.5-, 17-, 44.5-, and 47-kDa polypeptides, since these appear to be the most predictive of syphilis infection. We have observed cross-reactivity in the 30- to 40-kDa range in random donor specimens showing no reactivity with any of the major immunodominant epitopes (data not shown). Thus far, we have not encountered a problem of interference with nonsyphilitic specimens reacting with more than one of the four major *T. pallidum* antigens. Nevertheless, we must caution that the possibility of cross-reactions does exist, and a more complete evaluation of the specificity of the *T. pallidum* Western blot assay will require an extensive study with clinically defined, nonsyphilitic specimens, including sera from patients with other spirochetal infections, such as Lyme disease, leptospirosis, and periodontal disease (particularly with the elderly).

The ideal confirmatory test for syphilis should be fast, nonsubjective, sensitive, and specific. FTA-ABS (DS) has long been accepted in the United States as an excellent confirmatory test for syphilis, with a high degree of sensitivity. We believe that the Western blot assay with a whole-cell lysate of *T. pallidum* has been demonstrated to show sensitivity and specificity equal to or better than those of FTA-ABS (DS). The Western blot assay showed no equivocal reactivities with the specimens tested and had no difficulties with specimens containing elevated gamma globulin or ANA. As in FTA-ABS (DS), the Western blot strips can be standardized in large batches and stored frozen until needed. A minor disadvantage of the Western blot assay is that laboratories that are not already using the technology will incur a minor equipment expense. With Western blotting now being used in many laboratories for anti-human immunodeficiency type 1 and anti-human T-cell lymphotropic virus type I confirmation, the time may be right for syphilis confirmatory testing to move in that direction.

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REFERENCES

1. **Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136**:175–179.
2. **Buchanon, C., and J. Haserick.** 1970. FTA-ABS test in pregnancy: a probable false-positive reaction. Arch. Dermatol. **102**:322–325.
3. **Center for Disease Control.** 1977. Maintenance of *Treponema pallidum* (Nichols strain) in rabbits. Center for Disease Control, Atlanta.
4. **Hausser-Scientific Partnership.** 1972. Form DU90. Directions for use of Petroff-Hausser and Helber chambers. Hausser Scientific, Horsham, Pa.
5. **Hensel, U., H.-J. Wellensiek, and S. Bhakdi.** 1985. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting as a serological tool in the diagnosis of syphilitic infections. J. Clin. Microbiol. **21**:82–87.
6. **Hunter, E. F., M. R. Adams, L. H. Orrison, B. J. Pender, and S. A. Larsen.** 1979. Problems affecting performance of the fluorescent treponemal antibody-absorption test for syphilis. J. Clin. Microbiol. **9**:163–166.
7. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Tech. **1**:3–8.
8. **Kraus, S. J., J. R. Haserick, and M. Lantz.** 1970. Fluorescent treponemal antibody-absorption test reactions in lupus erythematosus. Atypical beading pattern and probable false-positive reactions. N. Engl. J. Med. **282**:1287–1251.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Larsen, S., C. Farshy, B. Pender, M. Adams, D. Pettit, and E. Hambie.** 1986. Staining intensities in the fluorescent treponemal antibody-absorption (FTA-ABS) test: association with the diagnosis of syphilis. Sex. Transm. Dis. **13**:221–227.
11. **Lukehart, S., S. Baker-Zander, and E. Gubish.** 1982. Identification of *Treponema pallidum* antigens: comparison with a nonpathogenic treponeme. J. Immunol. **129**:833–838.
12. **Mackey, D. M., E. Pride, J. Knox, and A. Scotti.** 1969. Specificity of the FTA-ABS test for syphilis: an evaluation. J. Am. Med. Assoc. **207**:1683–1685.
13. **Marchitto, K. S., S. A. Jones, R. F. Schell, P. L. Holmes, and M. V. Norgard.** 1984. Monoclonal antibody analysis of specific

antigenic similarities among pathogenic *Treponema pallidum* subspecies. Infect. Immun. **45**:660–666.

14. **Miller, J. N.** 1975. Value and limitations of nontreponemal and treponemal tests in the laboratory diagnosis of syphilis. Clin. Obstet. Gynecol. **18**:191–203.
15. **Millipore Corp.** 1987. Immobilon™ technical protocol 013. Human immunodeficiency virus (HIV) detection using electrotransfer (Western blot) to Immobilon™-P transfer membrane and enzyme immunoassay (EIA) development. Millipore Corp., Bedford, Mass.
16. **Monson, R. A. M.** 1973. Biologic false-positive FTA-ABS test in drug-induced lupus erythematosus. J. Am. Med. Assoc. **224**: 1028–1030.
17. **Norris, S. J., J. F. Aldereete, N. H. Axelsen, M. Bailey, S. A. Baker-Zander, J. B. Baseman, P. J. Bassford, R. E. Baughn, A. Cockayne, P. A. Hanff, P. Hindersson, S. A. Larsen, M. A. Lovett, S. A. Lukehart, J. N. Miller, M. A. Moskophidis, F. Muller, M. V. Norgard, C. W. Penn, C. V. Stamm, J. D. A. van Embden, and K. Wicker.** 1987. Identity of *Treponema pallidum* subsp. pallidum polypeptides: correlation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis results from different laboratories. Electrophoresis **8**:77–92.
18. **Public Health Service.** 1969. Manual of tests for syphilis. Publication 411, p. 15–22. U.S. Government Printing Office, Washington, D.C.
19. **Rosner, B.** 1982. Fundamentals of biostatistics, p. 159–162. Duxbury Press, Boston.
20. **Rosner, B.** 1982. Fundamentals of biostatistics, p. 294–303. Duxbury Press, Boston.
21. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
22. **Tuffanelli, D.** 1968. False positive reactions for syphilis. Arch. Dermatol. **98**:606–611.
23. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**:4406–4412.